APPENDIX A
PENDING CLAIMS SUBJECT TO EXAMINATION

100. (Three times amended) A formulation for therapeutic or diagnostic use comprising targeted gas-filled vesicles which comprise one or more membranes encapsulating an internal void that contains a gas selected from the group consisting of perfluorocarbons and sulfur hexafluoride, said membrane comprising a phospholipid, and being substantially free of crosslinked proteins and polymers, and further comprising a conjugate that comprises a lipid, a linking group, and a targeting ligand, wherein said linking group is a hydrophilic polymer that is covalently bound to both said lipid and said targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and wherein said targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor.

102. (Once Amended) A formulation according to Claim 100 wherein said lipid vesicles are selected from the group consisting of micelles and liposomes.

103. A formulation according to Claim 100 wherein said gas is derived, at least in part, from a gaseous precursor.

127. (Three times amended) A method for the therapeutic delivery *in vivo* of a bioactive agent comprising administering to a patient a therapeutically effective amount of a formulation which comprises, in combination with a bioactive agent, targeted gas-filled vesicles which comprise one or more membranes encapsulating an internal void that contains a gas selected from the group consisting of perfluorocarbons and sulfur hexafluoride, said membrane comprising a phospholipid, and being substantially free of crosslinked proteins and polymers, and further comprising a conjugate that comprises a lipid, a linking group, and a targeting ligand, wherein said linking group is a hydrophilic polymer that is covalently bound to said lipid and said targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and wherein said targeting ligand targets cells or receptors selected from the group consisting of

myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor.

194. (Once Amended) A formulation according to Claim 100 wherein said lipid vesicles comprise a phospholipid.

195. A formulation according to Claim 194 wherein said phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid.

196. A formulation according to Claim 195 wherein said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

197. A formulation according to Claim 196 wherein said phosphatidylcholine comprises dipalmitoylphosphatidylcholine.

198. A formulation according to Claim 195 wherein said phosphatidylethanolamine is selected from the group consisting of dipalmitoyl-phosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyldioleoyl-phosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine.

199. A formulation according to Claim 198 wherein said phosphatidylethanolamine comprises dipalmitoylphosphatidylethanolamine.

200. A formulation according to Claim 195 wherein said phosphatidic acid comprises dipalmitoylphosphatidic acid.

203. (Once amended) A formulation according to Claim 100 wherein said hydrophilic polymer comprises polyethylene glycol.

210. A formulation according to Claim 100 wherein said fluorinated gas comprises a perfluorocarbon.

211. A formulation according to Claim 210 wherein said perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

212. A formulation according to Claim 211 wherein said perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane.

213. A formulation according to Claim 212 wherein said perfluorocarbon gas comprises perfluorobutane.

214. A formulation according to Claim 103 wherein said gaseous precursor has a boiling point of greater than about 37°C.

215. A formulation according to Claim 214 wherein said gaseous precursor comprises a perfluorocarbon.

216. A formulation according to Claim 215 wherein said perfluorocarbon is selected from the group consisting of perfluoropentane and perfluorohexane.

217. A formulation according to Claim 100 wherein said targeting ligand is selected from the group consisting of proteins, peptides, saccharides, steroids, steroid analogs, bioactive agents and genetic material.

218. A formulation according to Claim 217 wherein said targeting ligand is selected from the group consisting of proteins, peptides and saccharides.

219. A formulation according to Claim 218 wherein said targeting ligand is selected from the group consisting of proteins and peptides.

220. A formulation according to Claim 219 wherein said targeting ligand comprises a peptide.

221. A formulation according to Claim 220 wherein said peptide comprises a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val.

222. A formulation according to Claim 219 wherein said targeting ligand comprises the sequence Arg-Gly-Asp.

223. A formulation according to Claim 100 wherein said receptors comprise the glycoprotein GPIIbIIIa receptor.

224. A formulation according to Claim 223 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of no greater than about 10^{-3} molar.

225. A formulation according to Claim 224 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of less than about 10^{-3} molar.

226. A formulation according to Claim 225 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-9} molar to less than about 10^{-3} molar.

227. A formulation according to Claim 226 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-7} molar to about 10^{-5} molar.

228. A formulation according to Claim 227 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of about 10^{-6} molar.

294. (Once Amended) A method according to Claim 127, wherein said lipid vesicles comprise a phospholipid.

295. A method according to Claim 294 wherein said phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid.

296. A method according to Claim 295 wherein said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

297. A method according to Claim 296 wherein said phosphatidylcholine comprises dipalmitoylphosphatidylcholine.

298. A method according to Claim 295 wherein said phosphatidylethanolamine is selected from the group consisting of dipalmitoyl-phosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyldioleoyl-phosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine.

299. A method according to Claim 298 wherein said phosphatidylethanolamine comprises dipalmitoylphosphatidylethanolamine.

300. A method according to Claim 295 wherein said phosphatidic acid comprises dipalmitoylphosphatidic acid.

303. (Once amended) A method according to Claim 127 wherein said hydrophilic polymer comprises polyethylene glycol.

310. A method according to Claim 127 wherein said fluorinated gas comprises a perfluorocarbon.

311. A method according to Claim 310 wherein said perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

312. A method according to Claim 311 wherein said perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane.

313. A method according to Claim 312 wherein said perfluorocarbon gas comprises perfluorobutane.

314. A method according to Claim 127 wherein said targeting ligand is selected from the group consisting of proteins, peptides, saccharides, steroids, steroid analogs, bioactive agents and genetic material.

315. A method according to Claim 314 wherein said targeting ligand is selected from the group consisting of proteins, peptides and saccharides.

316. A method according to Claim 315 wherein said targeting ligand is selected from the group consisting of proteins and peptides.

317. A method according to Claim 316 wherein said targeting ligand comprises a peptide.

318. A method according to Claim 317 wherein said peptide comprises a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val.

319. A method according to Claim 318 wherein said targeting ligand comprises the sequence Arg-Gly-Asp.

320. A method according to Claim 127 wherein said receptors comprise the glycoprotein GPIIbIIIa receptor.

321. A method according to Claim 320 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of no greater than about 10^{-3} molar.

322. A method according to Claim 321 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of less than about 10^{-3} molar.

323. A method according to Claim 322 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-9} molar to less than about 10^{-3} molar.

324. A method according to Claim 323 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-7} molar to about 10^{-5} molar.

325. A method according to Claim 324 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of about 10^{-6} molar.

326. A method according to Claim 127 further comprising the administration of a sufficient amount of ultrasound energy to induce rupture of said vesicles.

327. A method according to Claim 326 wherein said targeting ligand targets the glycoprotein GPIIbIIIa receptor.

328. A method according to Claim 327 wherein said glycoprotein GPIIbIIIa receptor is associated with a thrombus.

329. A method according to Claim 328 wherein the amount of said ultrasound energy is also sufficient to stimulate lysis of said thrombus.

331. (Once amended) A method according to Claim 329, wherein said lipid vesicles comprise a phospholipid.

332. A method according to Claim 331 wherein said phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid.

333. A method according to Claim 332 wherein said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

334. A method according to Claim 333 wherein said phosphatidylcholine comprises dipalmitoylphosphatidylcholine.

335. A method according to Claim 332 wherein said phosphatidylethanolamine is selected from the group consisting of dipalmitoyl-phosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyldioleoyl-phosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine.

336. A method according to Claim 335 wherein said phosphatidylethanolamine comprises dipalmitoylphosphatidylethanolamine.

337. A method according to Claim 332 wherein said phosphatidic acid comprises dipalmitoylphosphatidic acid.

347. A method according to Claim 329 wherein said fluorinated gas comprises a perfluorocarbon.

348. A method according to Claim 347 wherein said perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

349. A method according to Claim 348 wherein said perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane.

350. A method according to Claim 349 wherein said perfluorocarbon gas comprises perfluorobutane.

351. (Twice amended) A method according to Claim 329 wherein said targeting ligand is a peptide comprising a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1).

352. A method according to Claim 351 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of no greater than about 10^{-3} molar.

353. A method according to Claim 352 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of less than about 10^{-3} molar.

354. A method according to Claim 353 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-9} molar to less than about 10^{-3} molar.

355. A method according to Claim 354 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-7} molar to about 10^{-5} molar.

356. A method according to Claim 355 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of about 10^{-6} molar.